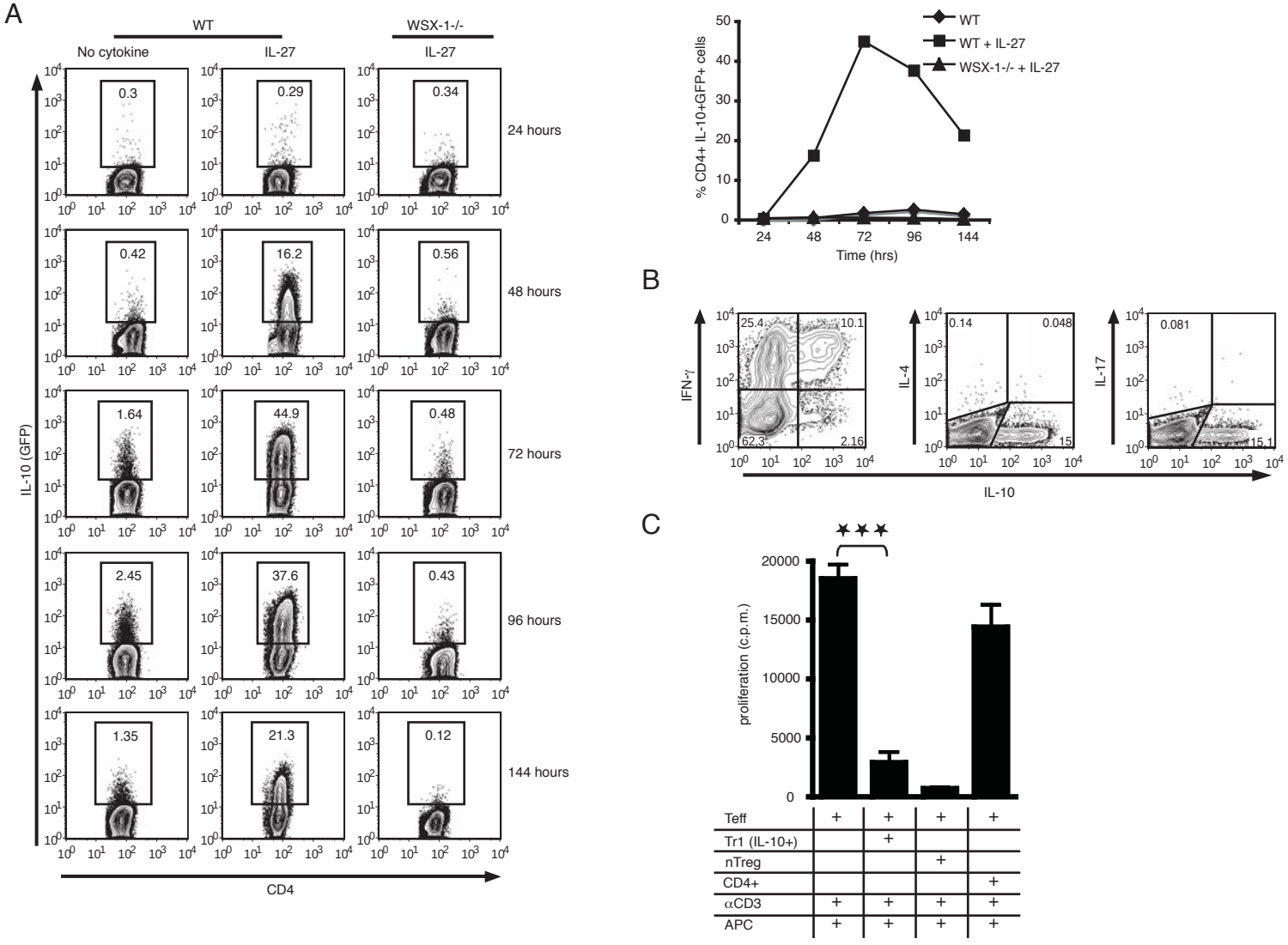
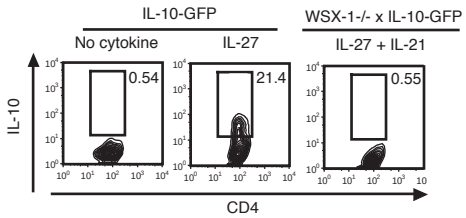
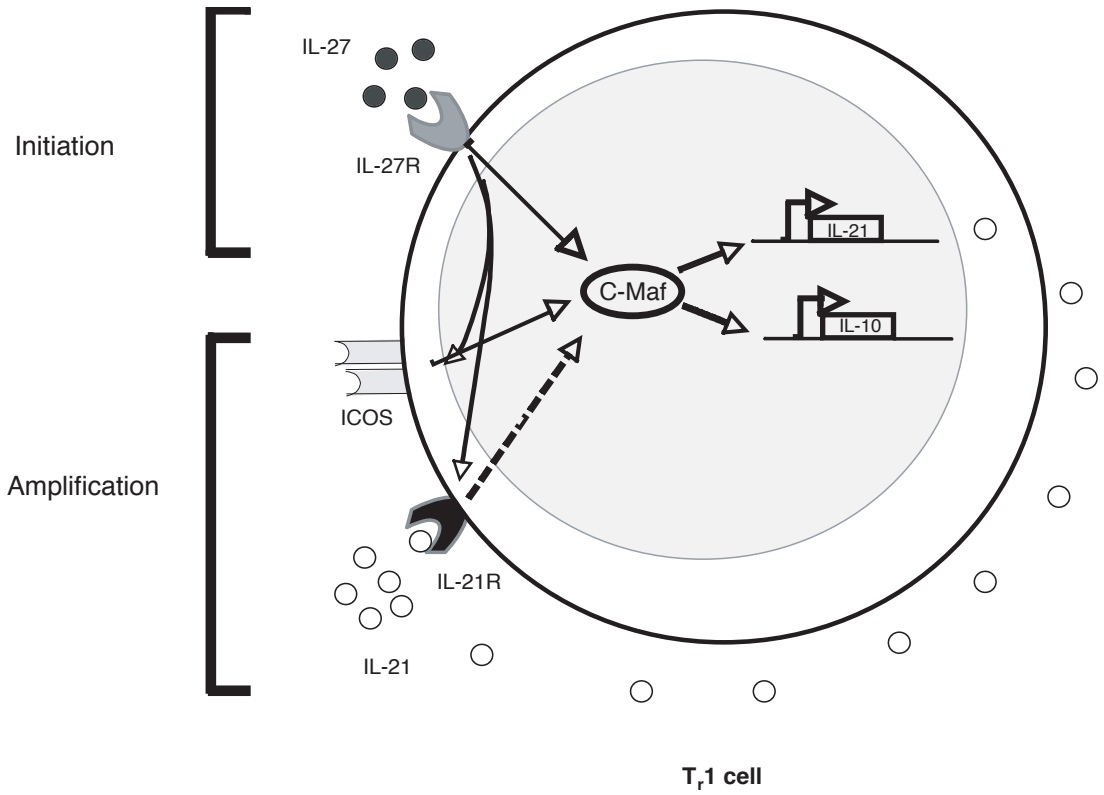


Supplementary Figure 1. **IL-27 induces IL-10-producing Tr1 cells that suppress T cell responses.** (A) Naïve CD4+CD62LhiCD25- T cells obtained from wild type IL-10-eGFP reporter ("Vert-X") or IL-27 ra-/- (WSX-1-/-) x Vert-X mice were differentiated in the presence of IL-27 and anti-TGF-β and examined at different time points following activation for the expression of IL-10.GFP+ T cells by flow cytometry. (B) Coexpression of IL-10 with IFN-γ, IL-4 or IL-17 was assessed by intracellular cytokine staining in T cells differentiated with IL-27 after 48 h; (C) Tr1 cells generated in the presence of IL-27 were tested for their suppressive capacity in an in vitro proliferation assay. A fixed number of naïve responder T cells (Teff, CD4+Foxp3- from Foxp3.gfp.KI mice) were cultured with Tr1 cells (CD4+IL-10.GFP+ from Vert-X mice) in the presence of irradiated syngenic splenocytes as APC and anti-CD3. Mean [3H] thymidine incorporation indicated as c.p.m. (+s.d.) in triplicate wells is shown. A positive control, nTreg (naïve CD4+Foxp3+ from Foxp3.gfp.KI mice) and a negative control, naïve CD4+ cells (CD4+) from Vert-X mice cultured without IL-27, were included. Tr1 cells significantly suppressed the proliferation of responder T cells (Teff) ($P<0.0005$).



Supplementary Figure 2. IL-21 cannot induce IL-10 in the absence of IL-27 signaling. IL-10.GFP expression as analyzed by flow cytometry in naïve T cells activated for 72 h, in the absence or presence of IL-27 from wild type IL-10-eGFP reporter ("Vert-X") mice and in the presence of IL-27 and IL-21 from IL-27R-/- (WSX-1) x Vert-X reporter mice.



Supplementary Figure 3. Induction of Tr1 cells by IL-27. IL-27 / IL-27R signaling induces c-Maf that directly transactivates IL-21 promoter. IL-21 amplifies Tr1 by acting as a growth factor for them. ICOS costimulatory molecule induced by IL-27 further increases c-Maf expression, IL-21 production and IL-10 transactivation.